REMARKS

Reconsideration and withdrawal of the rejections of the application are respectfully requested in view of the remarks and amendments herewith.

I. STATUS OF THE CLAIMS AND FORMAL MATTERS

Claims 1-2, 4-12 and 15-32 are now pending, and claims 1-2, 4-12, 15-17, and 22-25 are under examination. Claims 1, 4, 17-19 and 22-29 have been amended, and claims 3, 13 and 14 have been cancelled, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents.

No new matter is added.

It is submitted that these claims are in full compliance with the requirements of 35 U.S.C. §112. The amendments to the claims and the remarks herein are not made for the purpose of patentability within the meaning of 35 U.S.C. §§ 101, 102, 103 or 112; but rather the amendments and remarks are made simply to place the claims in better condition for examination and to correct typographical errors. Support for amended claim 1 can be found in the specification at page 3, line 75; page 8, line 176; page 13, line 296; page 22, line 479, and the example beginning at page 52, lines 1184; further support for amended claim 1 can be found in claim 3 as originally filed, and in Examples 8 (specifically Table 7) which describes varying concentrations of CTAB and incubation times of from 4 to 48 hours. In addition, support for the "specific activity" recitation in amended claim 1 can be found on page 12 of the specification as filed at lines 291 and 292.

II. THE REJECTIONS UNDER 35 U.S.C. §112 ARE OVERCOME

Claims 1-17 and 22-25 were rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one of skill in the art that the inventors had possession of the application at the time of filing. And, claims 1-17 and 22-25 were rejected under 35 U.S.C. §112, first paragraph, because the specification allegedly is not enabling for a method for the specific release of any POI. The rejections are respectfully traversed.

35 U.S.C. §112, first paragraph, requires that the specification describe how to make and use the invention. 35 U.S.C. §112, first paragraph, recites, in pertinent part:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same[.]

A patent claim is invalid if it is not, *inter alia*, supported by an enabling disclosure. The test for enablement requires a determination of whether any person skilled in the art can make and use the invention without undue experimentation. *See In re Wands*, 858 F.2d 731, 8 U.S.P.Q.2d 1400, (Fed. Cir. 1988). The factors involved in determining whether there is sufficient evidence to support a finding of enablement include, among others, (1) the breadth of the claims, (2) the nature of the invention, (3) the state of the prior art, (4) the level of one of ordinary skill, (5) the level of predictability in the art, (6) the amount of direction provided by the inventor, (7) the existence of working examples, and (8) the quantity of experimentation needed to make or use the invention based on the content of the disclosure. *See Wands*, 858 F.2d at 737, 8 U.S.P.Q.2d at 1404.

It is respectfully submitted that the present application satisfies both the written description and enablement requirements as described above.

Turning first to the written description rejections, the Office Action stated that the claims are drawn to a method of making a genus of POIs which "encompasses unlimited number of species having wildly different structures and functions." Office Action at 4. The Office Action additionally states that the specification lacks "[s]tructural features that could distinguish compounds in the genus from others in the protein classes" and that "trial and error experimentation" is required for "the development of an appropriate purification scheme for a specific protein". Office Action at 4.

Applicants respectfully disagree with these assertions. Initially, the invention as presently claimed does not "encompasses unlimited number of species". Rather, the present invention relates only to recombinant proteins of interest from a bacterial, yeast or fungal cell.

The description as filed describes in detail the components of a membrane extracting composition, as well as the conditions under which it may optimally be used. The Examiner's attention is respectfully directed to the specification at, for example, page 13, lines 304 to 310 and the following disclosure up to and including page 21. The specification as filed discloses,

inter alia, expression of proteins in yeast host organisms (page 25) and provides detailed instructions on how to extract these expressed proteins from yeast cells, using quaternary ammonium compounds, examples of which are set out at pages 13 to 18. The conditions which are required for releasing the protein from the yeast cells are discussed generally at pages 19 to 21, which describe the concentration of the quaternary ammonium compound as well as the temperature and pH at which these may be used, such that sufficient disclosure of the conditions necessary to the practice of the present invention is provided.

The Office Action further states that although the claims recite "conditions sufficient for the specific release of the POI", the present application "does not provide a correlation between the specific POI and said conditions". Office Action at 4-5. Again, the present application provides general guidelines as to the quarternary ammonium compounds to be used, including those concentration levels that are most effective, as well as guidelines as to the appropriate pH, temperature, and incubation times.

Taken in combination with the detailed experimental protocols set out in the Examples, one of skill in the art would find that the claimed methods were sufficiently described and that the inventors were indeed in possession of the invention at the time of filing. For instance, Examples 1 to 3 describe the expression of recombinant hexose oxidase (HOX) in *Hansenula polymorpha*, while Example 4 shows that the basic extraction method works. Examples 5 and 6 compare extraction using different membrane extracting compositions. Example 7 shows that protein is extracted without contaminating compounds, while Example 8 explores the effects of temperature on protein extraction. Examples 9, 10 and 11 compare various compounds and their effect on the extraction of protein. Examples 12, 13 and 14 demonstrate that it is possible to scale up the process for commercial use. Example 15 discloses the use of Triton X-100 detergent on the extraction of hexose oxidase from *H. polymorpha*, while Example 16 shows almost complete extraction of hexose oxidase with 0.4% CTAB. High throughput screening (HTS) is described at Example 17, while Examples 18 to 23 describe a number of comparisons between CTAB extracted HOX and mechanically extracted HOX. Furthermore, Examples 24 to 27 describe the extraction of another protein, glucan lyase from H. polymorpha, using LTAB detergent, while Examples 28 and 29 compare extraction of glucan lyase in *H. polymorpha* and *P. pastoris*, and between mechanical and chemical recovery methods. Example 33 shows that glucan lyase can be extracted from *H polymorpha* on an industrial scale.

Clearly, Applicants performed myriad experiments with varied protocols and proteins of interest while arriving at the invention, all of which therefore provide invaluable guidance to others who desire to make and use the invention, including the delineation of parameters such as incubation times, temperatures, etc. that provide the user with a successful extraction of the protein of interest.

As another basis for rejection, the Office Action states that the specification fails to teach any representative species of hexose oxidase other than *Chondrus crispus*, nor are identifying characteristics of other species provided. Applicants respectfully submit that this is a mischaracterization as Examples 24 to 33 show the expression of another protein (namely, a-1,4-glucan lyase) from the yeast *Hansenula polymorpha*. The expressed protein was extracted using LTAB, a quaternary ammonium compound by a method as described and claimed in the present application. It is clear from these Examples that the expressed and extracted glucan lyase has a higher specific activity when extracted by the claimed methods as opposed to mechanical extraction, as required by the claims.

Specifically, the expressed and extracted glucan lyase has a specific activity as high as 9.27 mol 1,5-anhydrofrucose/min.ml. In contrast, Yu et al. (1999 - mentioned in the specification at page 106, lines 2097 to 2102) reported expression and extraction of glucan lyase by secretion by means of a signal peptide, with a low specific activity and yield of 0.7 mol 1.5-anhydrofrucose/min.mg protein. Thus, these examples demonstrated that extraction by quaternary ammonium compounds of intracellularly expressed recombinant proteins therefore results in a higher yield than the prior art methods, as is required by the claims and which is clearly described in the specification for proteins besides hexose oxidase.

Furthermore, as amended herein the claims now require that the protein of interest be a recombinant protein, and that the protein be from a bacterial, yeast or fungal cell, thereby providing further identifying characteristics of those proteins which may be extracted by the claimed methods.

The Office Action additionally alleges that a single method is provided in the present application, which is insufficient to place one in possession of all of the species within the claimed genus of POIs. Again, applicants respectfully disagree with this contention.

Applicants respectfully submit that the process as claimed is general in nature, and may be applied to a number of proteins with modifications that are commonplace to those of skill in

the art and which require no undue experimentation. Indeed, the present specification, as well as the claims themselves, provide guidelines as to modifications of the claimed methods; for instance, effective ranges of incubation times and temperatures are provided, as are quarternary ammonium compounds that may be used and the range of concentrations at which the compounds should be used.

For instance, the Examples describe the expression and extraction of hexose oxidase in *H. polymorpha*, as well as a number of HOX enzyme mutants (see Example 17), in addition to the extraction of glucan lyase. Example 20 further describes expression and isolation of a number of IL- 1 receptor antagonists, as does Example 21. The Examples fully demonstrate both the range of incubation times and the useful concentrations of the quarternary ammonium compounds which can be used in the practice of the claimed method. For instance, Table 7 on pages 66 and 67 (Example 8) depicts a series of experiments conducted at different temperatures and concentrations of CTAB at 4, 8, 24, 31 and 48 hours. The Examples describe this experiment in which cells are incubated with the membrane extracting composition for 7, 17, 19, 20, 22, 23, 24, 26 and 48 hours (see Example 8 specifically, and Examples 7-10 generally, which compare various quarternary ammonium compounds and concentrations).

Furthermore, review of the Examples, including specifically Tables 16 and 17, demonstrates that the extracted proteins are clearly seen to have higher specific activities compared to proteins extracted by mechanical means, as is required by the claims and which improved results are provided by the presently claimed processes.

Therefore, as has been demonstrated above, the present specification provides sufficient written support for all elements of the claims, including for the extraction of proteins other than hexose oxidase and for variations of the claimed method due to substitutions of the quarternary ammonium compound, variations in concentration of the compound, or of the incubation time or temperature. Consequently, reconsideration and withdrawal of the written description rejections based on 35 U.S.C. §112, first paragraph, is respectfully requested.

Turning now to the enablement rejection, the Office Action alleges that the specification does not enable a method for the specific release of any POI under "sufficient conditions". The Office Action alleges that there is no guidance in the specification as to how such sufficient conditions are determined "given that proteins differ widely in properties." Office Action at 6 and 7. Applicants respectfully disagree.

Initially, it is noted that the Office Action focuses on the "specific release" of any POI. Indeed, the phrase "specific release" was additionally the subject of a rejection under 35 U.S.C. §112, second paragraph (*see infra*). The amendment herein has removed this phrase from the claims, such that to the extent the enablement rejection turns on lack of enablement for the "specific release" of any POI, the rejection is now moot. As the amendment to claim 1 instead now recites the increased "specific activity" of the POI, it is respectfully submitted that this phrase poses no impediment to enablement. Specific activity is well understood in the art as a measure of the enzyme activity per unit mass of enzyme, and is a valid measure of the improvements that result from the use of the presently claimed method in contrast to mechanical means of protein extraction.

Furthermore, claim 1 now specifies that the membrane extracting composition comprises a quaternary ammonium compound at a concentration of between 0.05% to 0.6% by weight, and that it is contacted with a cell for between 4 to 48 hours. And, claim 1 is now limited to recombinant proteins of interest from a bacterial, yeast or fungal cell. As a result, the claims now define a specific type of protein to be extracted, from specific types of cells, by a specific method, all of which is fully demonstrated in the specification, as described above.

Indeed, any experimentation and optimization that the skilled artisan might need to engage in would be routine trial and error experiments; such simple endeavors pose no unnecessary burden on the skilled person and are within the scope of skilled artisans' daily activities. The specification as filed describes the invention in more than adequate detail, as outlined above, such that a skilled artisan having the specification would encounter no difficulty at all in performing the invention as claimed.

Consequently, when the present invention is viewed in light of the *Wands* factors, it is clear that the breadth of the claims is sufficiently appropriate based on the method provided, the level of one of ordinary skill is high, the level of predictability in the art is high given the knowledge of this in the field and the specific guidelines provided in the specification, the amount of direction provided by the inventor is high, working examples exist in the specification and provide variations of the method within the scope of the claims, and the quantity of experimentation needed to make or use the invention based on the content of the disclosure is low as a result of the described elements, i.e., the identification of quarternary ammonium

compounds, guidance as to the concentrations at which such compounds should be utilized, and guidance as to incubation times.

For all of these reasons, the presently pending claims are fully enabled by the specification as filed, and reconsideration and withdrawal of the enablement rejection under 35 U.S.C. §112, first paragraph, is respectfully requested.

Claims 1-17 and 22-25 were rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particular point out and distinctly claim the subject matter of the invention. The rejection is respectfully traversed.

Specifically, the Office Action stated that claim 1(c) recited the term "specific release" which is allegedly not commonly accepted in the art. Furthermore, the meaning of "and in a soluble form" was considered unclear. Applicants respectfully submit that the claims have been amended to remove the word "specific" and that claim 1 has been amended to remove the word "and". Applicants respectfully note that rather than claim 1 referring to "specific release", claim 1 has been amended herein to specifically require that the released recombinant protein of interest has a specific activity higher than when the protein of interest has been extracted by mechanical means. It is respectfully submitted that the term "specific activity" is well understood in the art as a measure of the enzyme activity per unit mass of enzyme, and is therefore clear and definite.

The Office Action also stated that the word "preferably" in claim 13 rendered the claim indefinite. As claim 13 has been cancelled herein, the rejection is now moot as to this claim.

Finally, claims 22-25 were rejected due to the phrase "variant, homologue, derivative" as the terms allegedly do not have an art accepted meaning. Although Applicants disagree with this statement, in order to advance the prosecution of the present application, the claims have been amended to remove the phrase.

All of the rejections under 35 U.S.C. §112, second paragraph, are rendered moot by the amendments herein. Accordingly, reconsideration and withdrawal of the rejections under 35 U.S.C. §112, second paragraph, is respectfully requested.

III. THE ART REJECTIONS ARE OVERCOME

Claims 1-12 were rejected under 35 U.S.C. §102(b) as allegedly anticipated by Sundhey et al. The rejection is respectfully traversed.

Applicants respectfully remind the Examiner that a two-prong inquiry must be satisfied in order for a Section 102 rejection to stand. First, the prior art reference must contain all of the elements of the claimed invention, *see Lewmar Marine Inc. v. Barient Inc.,* 3 U .S.P.Q.2d 1766 (Fed. Cir. 1987), and, the single prior art reference must contain an enabling disclosure, *see Chester v. Miller,* 15 U.S.P.Q.2d 1333, 1336 (Fed. Cir. 1990).

The Office Action states that Sundhey teaches "the extraction of membrane proteins of different molecular weights from goat testiculuar cells using CTAB." Office Action at 9.

In contrast, the present claimed invention relates to methods for releasing a soluble or membrane associated intracellular **recombinant protein** of interest (POI) **from a bacterial, yeast or fungal cell**. Clearly, the buck spermatozoa of Sundhey is outside the scope of the bacterial, yeast or fungal cells used in the presently claimed method, and the membrane protein extracted by Sundhey is outside the scope of recombinant protein obtained by the claimed methods.

Furthermore, the methods of obtaining the proteins differs between Sundhey and the claimed method. Sundhey requires the use of detergents at 1% concentration during an incubation period of 1 hour. In contrast, the presently claimed method requires the use of a quarternary ammonium compound at a concentration of between 0.05% to 0.6% by weight, and an incubation period of between 4 to 48 hours. Clearly, the detergent concentration and incubation period utilized by Sundhey is outside the scope of the claims as presented herein, such that Sundhey fails to contain all of the elements of the claimed invention. For this reason, the rejection is improper and must be withdrawn.

Furthermore, neither does Sundhey provide any motivation or suggestion to modify its teachings to arrive at the present invention. Sundhey provides no suggestion or teaching that recombinant proteins could be extracted using the method described, let alone that the method could be applicable to cells other than buck spermatozoa. In particular, Sundhey provides not indication or suggestion that the reported method could be applied to bacterial, yeast or fungal cells. Furthermore, Sundhey provides no teaching or suggestion that would motivate one of skill in the art to lower the concentration of a quarternary ammonium compound to 0.05% to 0.6% with any expectation of success; nor does Sundhey provide any motivation to increase the incubation period at least four times over to arrive at an incubation time of between 4 to 48 hours. Therefore, Sundhey additionally fails to render the present claims obvious as Sundhey

provides no teaching or suggestion of the claimed method, nor does Sundhey provide any motivation to modify the method of Sundhey to arrive at the claimed method with any expectation of success.

Consequently, reconsideration and withdrawal of the rejections under 35 U.S.C. §102(b) is respectfully requested.

REQUEST FOR INTERVIEW

If any issue remains as an impediment to allowance, prior to issuance of any paper other than a Notice of Allowance, an interview, is respectfully requested, with the Examiner his supervisor, and, the Examiner is respectfully requested to contact the undersigned to arrange a mutually convenient time and manner for such an interview.

CONCLUSION

I n view of the amendments, and remarks herein, the application is in condition for allowance. Reconsideration and withdrawal of the rejections of the application, and prompt issuance of a Notice of Allowance, is respectfully requested.

Respectfully submitted,
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